

# SOME EXPERIMENTS UPON TEMPERATURE ACCLIMATIZATION AND RESPIRATORY METABOLISM IN FISHES<sup>1</sup>

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## *Introductory, Procedure, etc.*

In a preliminary communication from this laboratory (Sumner and Wells, 1935), experiments were described in which fishes of varying temperature history were subjected to a number of lethal agents and anesthetics. The most important conclusions drawn from these experiments were stated as follows:

"Fishes which had been kept at widely different temperatures, when tested at these temperatures, were found to differ markedly in the rate of their visible respiratory movements. . . . Other things equal, these are higher at higher temperatures than at lower ones.<sup>2</sup> . . . When fishes which had become acclimatized to high and low temperatures, respectively, were transferred to a common temperature (usually an intermediate one), those from the warmer water displayed a lower respiratory rate and a lower susceptibility to the anesthetics and lethal agents than those from the colder water. Thus the differences in physiological activity which had been originally induced by acclimatization to these differing temperatures were completely reversed when the fishes were brought to a common temperature. After this transfer, it was the former "warm" fishes which had the lower respiratory rate etc. and the former "cold" fishes which had the higher. Fishes kept at intermediate temperatures remained intermediate.

"Acclimatization to a high temperature appears to consist, in part at least, of a change whereby an initial great increase in metabolic rate is followed by a regulative process, with continued sojourn in warm water, and, conversely, acclimatization to a low temperature involves a similar process working in the opposite direction."

Somewhat earlier N. A. Wells (1935a), from actual determinations of oxygen consumption in *Fundulus parvipinnis*, had concluded that

<sup>1</sup> Contributions from the Scripps Institution of Oceanography, New Series, No. 9.

<sup>2</sup> The further statement in the original text relative to higher susceptibility to urethane at higher temperatures has not been substantiated by our later work.

"the rate of metabolism of fishes at any given temperature is dependent upon the temperature of the water to which they have been [previously] acclimatized."

That the resistance of a living organism to cyanides and some other poisons varies inversely with the temperature was established by Child for planarians and other invertebrates (Child, 1913). Geppert (1889) is said to have proposed the view, now generally accepted, that the cyanides act by preventing the tissues from utilizing oxygen.

The occurrence of a certain amount of physiological adaptation in the effects of temperature upon metabolic rate was pointed out by Miss Behre (1918), working upon *Planaria dorotocephala*. In a discussion of experiments in which the periods of acclimatization ranged from three days to three months, she states: "Worms tested immediately after they have been put into a higher temperature than that at which they have been living for a shorter or longer time show greater susceptibility to cyanide than those which have been living indefinitely at a higher temperature; those tested immediately after they have been put into a lower temperature than that at which they have been living for a shorter or longer time show a lower susceptibility to cyanide than those which have been living indefinitely at the lower temperature."

Our discussion has thus far related to the effect of temperature upon metabolic rate, and the only result of acclimatization thus far considered has consisted in a fall in this rate, following an initial rise, or a rise following an initial fall. These changes in metabolic rate have been detected by observing the relative susceptibility to KCN or certain other poisons. Another type of acclimatization has to do with the effects of subjection to moderately high or low temperatures upon subsequent resistance to heat and cold, when these are used as lethal agents. That this latter class of effects is quite distinct from the former one will appear probable from facts to be reported below.

Among the earlier experiments in acclimatizing vertebrate animals to relatively high temperatures, those of Davenport and Castle (1895) are so familiar that a brief reference to them is sufficient. By subjecting toad tadpoles for four weeks to 24°–25°, instead of 15°, at which the controls were living, the temperature necessary to produce heat-rigor was raised by 3.2° C.

Loeb and Wasteneys (1912) conducted some highly interesting experiments upon *Fundulus* (presumably *F. heteroclitus*). These experiments were largely concerned with the effects of various salts upon heat resistance. But the authors also sought to determine the maximum temperature which the fishes could tolerate, if transferred suddenly from their natural medium (10° to 14° at the time); "how long it

takes to immunize the fish against the harmful effects of a sudden transfer to a temperature of 35° C"; how long this immunity would last after return to cooler water, and some other matters. Certain of the results of Loeb and Wasteneys will be referred to later. For the most part, their paper does not report the numbers of fishes employed (where stated, they are very small), nor the variability of the individual records, so that the reader is left in doubt as to the statistical certainty of some of the results.

A rather extensive series of experiments on temperature acclimatization was conducted by Hathaway (1928) upon several species of fresh-water fishes as well as upon toad tadpoles. Hathaway's procedure consisted chiefly in keeping the animals for various periods at 10° and 30°, and then determining their mortality rate throughout various periods (1 minute to 24 hours) at temperatures ranging from 30° to 40°. "Continued exposure to high or low temperatures progressively raised or lowered the limit of tolerance of each species," a statement which is abundantly supported by his tables and histograms. No attempt was made, however, to relate the period of acclimatization in any quantitative way to the resulting degree of resistance.

The results herein reported are the outcome of experiments conducted by the authors throughout about a year past, in the course of which nearly three thousand fishes were employed. Except in one minor experiment, these fishes were gobies of the species *Gillichthys mirabilis* Cooper.

Our experimental material was kept in tanks of running sea-water at constant temperatures.<sup>3</sup> Tanks were maintained throughout at 10°, 20°, and 30° C., and for part of the time others were maintained at 15° and 25°. Prior to experimental treatment at high or low temperatures, all fishes were kept for some weeks or months at the intermediate temperature (20°). In some of our tables and charts, fishes from the 10°, 20°, and 30° tanks are designated as "C," "I," and "W," respectively.

The problems which have concerned us fall into two main groups as indicated in the preliminary discussion: (1) the effects of the subjection of fishes to higher or lower temperatures upon their respiratory metabolism; and (2) the effects of this sort of conditioning upon their

<sup>3</sup> Except on infrequent occasions, the temperatures were held to within 0.5° of the figures stated, by thermostatic control or otherwise. Owing to failure in the control mechanisms, water in the 10° tank occasionally exceeded or fell below this figure by several degrees. The temperature of the 30° tank was less subject to such perturbations, though it fell some degrees below the required temperature on several occasions. It never rose above this, however, except very early in the course of these experiments.

subsequent resistance to heat or cold, when these were employed as lethal agents.

(1) Some of the relations between this sort of temperature conditioning and respiratory metabolism were investigated by N. A. Wells (1935*a*, 1935*b*, 1935*c*) in this laboratory some years ago, through the direct measurement of oxygen consumption in *Fundulus parvipinnis*. In the present studies, no such direct measurements have been made. We have, however, tested some of these relations abundantly in other ways.

For this purpose, fishes of varying temperature history have been subjected to two principal lethal agents: KCN (0.001 M in sea water) and boiled sea water, containing about 5 per cent of the oxygen normally present. Since death from cyanide, as already stated, is due, in part at least, to asphyxiation, the rate of death from either of these lethal agents may be taken as a measure of respiratory metabolism.

In using each of these agents, our tests were of two sorts. The fishes were either (*a*) tested at the temperature, high or low, to which they had been acclimatized; or (*b*) they were tested after they had been returned for varying periods to the water of intermediate temperature (20°) from which they had been taken at the commencement of the experiment. In the latter case, the relations were also investigated between the duration of conditioning and the duration of the persistence of the induced effect.

In a limited number of experiments, the fishes of various temperature histories were subjected to the anesthetic urethane. The results from these experiments were less intelligible than those from other lethal agents, and the relation, earlier suggested (Summer and Wells, 1935), between metabolic rate and susceptibility to this drug, now seems rather doubtful.

(2) Under the second head, we have chiefly sought to determine (*a*) the relation between the duration of previous conditioning and the extent of the subsequent resistance to heat (i.e. how high temperatures were lethal to the fishes), and (*b*) the relation between the duration of conditioning and the persistence of the effect, when the fishes were returned for varying periods to their original temperature.

In these experiments with lethal heat, our procedure has consisted in placing small lots of the fishes under comparison simultaneously in separate screen cages, immersed in a common tank. In any given set of experiments, the temperature at the outset was brought to the same level (most frequently 37.6°), before introduction of the fishes. It was thereafter raised at an average rate of about 0.1° in two minutes. Record was kept of the moment when each fish succumbed, and of the



temperature of the water (to  $0.1^{\circ}$  or less) at that moment. Thus, it is evident that the temperatures recorded as lethal and the times recorded as the duration of resistance have no absolute significance. Their significance is entirely relative to the conditions of these experiments. In most of our experiments, there was little or no overlapping between lots of fishes having different temperature histories. This, although the mean lethal temperatures for such lots might differ by less than one degree.

Some tests were also made with a view to discovering the causes of certain unaccountable differences in our experimental results from lots of fishes which might have been expected to agree completely with one another. In general, none of the suspected causes were found to be responsible for these incongruities. The possible influence of differences in size, for example, was tested, despite our customary endeavor to select fishes of the same mean size for two lots under comparison. As will be pointed out below, size, within the range of differences which concern us here, could not have been a responsible factor in the matter, although size differences of sufficient magnitude were found to affect the respiratory rate and the rate of death in boiled water.

We likewise tested the possible effects of differences in the recency of feeding, and of minute differences in the concentration of the KCN solution. It was found that recency of feeding—even feeding within 24 hours—did not affect the resistance of the fishes to cyanide, while differences in the concentration of the solution as great as 2 per cent had no recognizable effect.

In all of our experiments, the value to be determined was the relative resistance of a given lot of fishes to the lethal agent or anesthetic which was employed. This resistance was measured by the time which elapsed before each individual "succumbed." It was consequently necessary to adopt a uniform criterion of "succumbing," in other words, a definite end-point which could be regarded as comparable throughout a given series.

The criterion adopted was necessarily different for the different types of experiment. In the case of lethal heat, the matter was simple. The end-point adopted was the time when the fish ceased to make any respiratory movements or other visible movements, spontaneous or induced. This time could ordinarily be recorded with an accuracy of less than half a minute. The figure recorded did not, however, necessarily represent the time of actual death, since many of these individuals recovered if transferred promptly to cooler water.

Where extreme cold was the "lethal" agent employed, a satisfactory end-point was more difficult to detect, since ready responsiveness to



sharp mechanical shocks persisted long after all spontaneous movements ceased. Although our criterion was changed somewhat from one experiment to another, it was constant within any one experiment, so that some of the results are highly instructive.

In experiments involving asphyxiation (KCN and boiled water) the actual death of the fish was revealed by a more or less prolonged convulsive seizure, which was readily recognizable in most cases. More often, this commenced rather dramatically, after some minutes of complete quiescence, beginning with a vigorous flexure of the body, and passing into a characteristic series of quivering and twitching movements of the mandible, opercula and fins. The length and intensity of this convulsion were inversely proportional to the temperature, its duration in KCN solution averaging about 6 minutes at 10°, 3 minutes at 20°, and 1 minute at 30°. At the latter temperature indeed, it was frequently so brief or so inconspicuous that it could not be observed at all. The time of the commencement of these death throes has been adopted as the end-point in experiments with KCN and boiled water.

TABLE I

Mean times of survival in KCN at various temperatures, after three days accommodation to these, and relative rates of respiratory metabolism on which KCN curve in Fig. 1 is based. The last figures are the quotients obtained by dividing the reciprocals of the various times by the reciprocal of the time at 10°.

Number of Fishes	Temperature of Acclimatization (±)	Actual Temperature in Bowls during Experiment	Times of Survival		Relative Metabolism
			Mean	Range	
20	°C. 10	°C. 10.7	308.5	241-375	1.0
19	15	15.6	184.0	153-221	1.7
20	20	20.4	109.2	89-139	2.8
17	25	25.0	64.4	56-78	4.8
20	30	29.5	42.4	34-52	7.3

In experiments with urethane, choice of an end-point was a more difficult matter, especially at low temperatures, a fact which detracts from the value of many of our experiments with this drug.

In addition to records of their relative resistance to lethal agents and to urethane, we have records of the rate of the respiratory rhythm of many of our lots of fishes. The value of these last, though real, is limited, (1) owing to the responsiveness of the respiratory rate to various unavoidable sensory stimuli, and (2) owing to the fact that there is another "dimension" to the respiratory movements—depth as

well as frequency. It is likely that the former may influence the amount of water passing through the gill-chambers as much as the latter, which alone can be recorded.

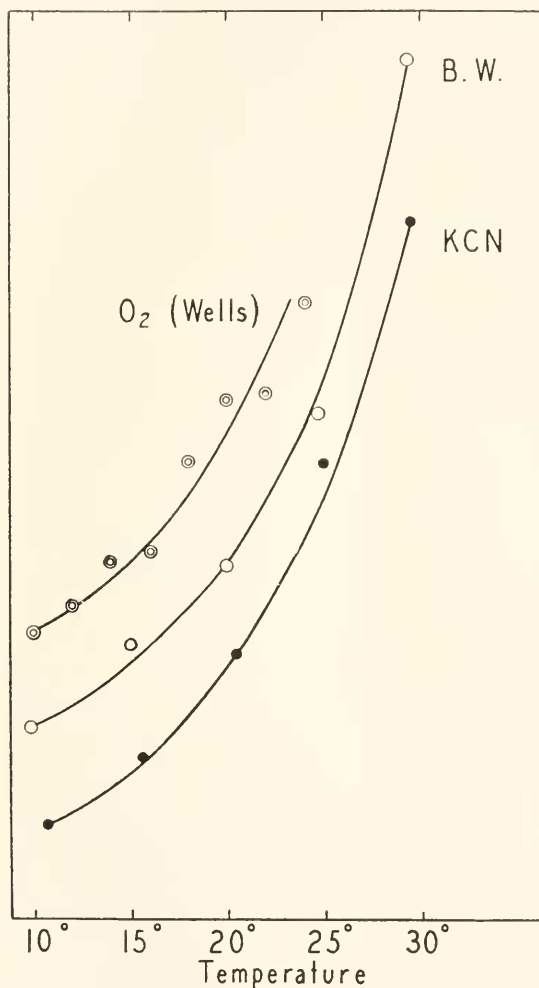


FIG. 1. Correlations between temperature and respiratory metabolism. The values for KCN and for boiled water (*B.W.*) are obtained in each case by dividing the reciprocals of the mean times of death at the various temperatures by the reciprocal of the time at 10° (equivalent to dividing mean time of death at 10° by each of the others). The upper curve is based upon measurements of oxygen consumption by N. A. Wells (1935, Table II (means of Experiments 4 and 5). Each of these curves is based upon relative values only. Their separate positions on the vertical scale have no significance.

The more significant results of all these experiments may be summarized as follows:

#### POTASSIUM CYANIDE

(1) Death in KCN was speedy in direct proportion to the temperature of the medium, fishes dying far more rapidly at a high temperature than at a low one. This fact is altogether in accordance with expectations, since the oxygen requirements of fishes and other poikilotherms are known to rise with increasing temperature.<sup>4</sup> When the reciprocals of the times of death are plotted against temperature, using the value at 10° as unity, a fairly exact logarithmic series is shown, of a form similar to that shown when the known data of oxygen consumption are similarly plotted (Table I, Fig. 1).<sup>5</sup> It will be seen that the  $Q_{10}$  lies between 2.6 and 2.8.

(2) A low correlation exists between the duration of acclimatization to high or low temperatures and the degree of resistance to KCN, at those temperatures. When fishes which had been subjected to 30° for a few hours, and for various numbers of days, up to a month or more, were compared in KCN at this temperature, the results were highly variable, though statistical differences of reasonable certainty were evident when all of the results were considered. From Table II

TABLE II

Relation between duration of acclimatization in 30° and resistance to KCN at the latter temperature. (Mean times in minutes).

Duration acclim.....	½ hr.-5 hrs.	1 day	3 days	5 days
Number of fishes.....	76	52	60	10
Length of resistance.....	34.67±0.25	41.79±0.42	44.52±0.53	47.90
Duration acclim.....	10-11 days	22-42 days		
Number of fishes.....	26	42		
Length of resistance.....	50.85±0.67	48.36±0.66		

it is evident that a progressive increase of resistance occurred, commencing with the briefest periods of acclimatization.

<sup>4</sup> It is possible of course, that the results with KCN may be influenced to some extent by the effects of temperature on diffusion, or on the "coefficient of distribution," or some other physical factor (cf. discussion by Child, 1913), though this could hardly affect the phenomena of reversal at a common temperature.

<sup>5</sup> Similar curves representing the correlation between temperature and metabolism have been published by Krogh (1916), Benedict (1932) and doubtless others.

This situation may be explained on the supposition that the initial rise in respiratory metabolism, resulting from the abrupt transfer to a higher temperature, is followed by an adjustment, in which the metabolism gradually falls again (Fig. 2). That it never even approximately reaches its former level, at least within the time limits of our experiment, is obvious from an inspection of the table and graphs.

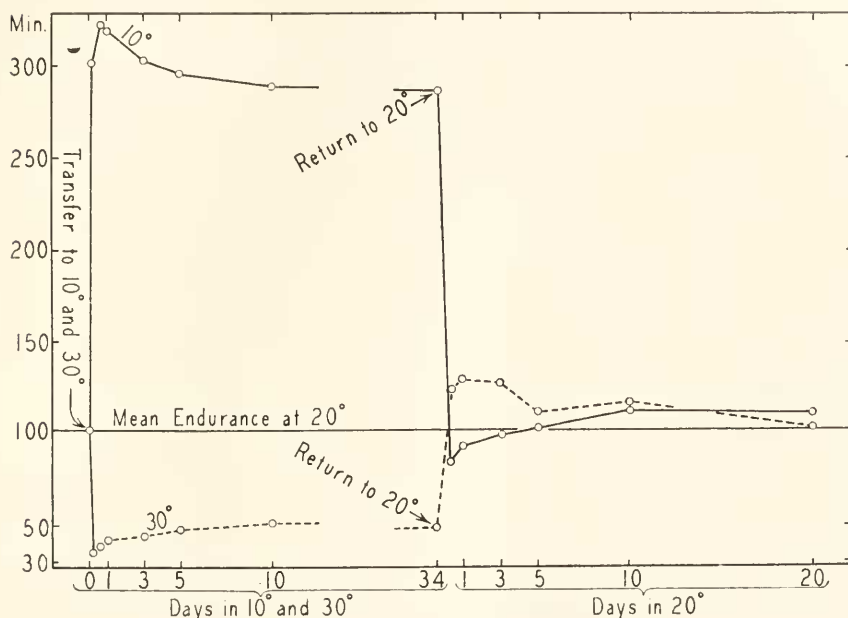


FIG. 2. Relations between duration of acclimatization in  $30^{\circ}$  and  $10^{\circ}$  respectively, and resistance to KCN at these temperatures. The  $30^{\circ}$  curve is based upon data in Table II, the  $10^{\circ}$  curve upon data in Table III. The line representing "Mean Endurance at  $20^{\circ}$ " (102 minutes) is based on all of the fishes (100) for which reliable figures at this temperature are available. Abscissas represent (left) days of acclimatization in  $10^{\circ}$  and  $30^{\circ}$  water, and (right) days following return to original temperature ( $20^{\circ}$ ). Ordinates represent survival time in minutes.

A similar, though converse, situation was to be expected when fishes were transferred to water of lower temperature instead of higher. An initial fall in respiratory metabolism (increase of resistance to KCN) would occur, followed by a compensatory rise, manifested in diminishing resistance. This, indeed, is what we have observed, though the variability of the individual figures is even greater than in the case of the warm water series, and the statistical probability of the successive steps is correspondingly less (Table III). A further difference be-

tween the "warm" and "cold" series is the fact that the maximum resistance of the latter (peak of the curve) is not attained as promptly as is the minimum resistance of the former, so that a further rise occurs within the period of our test, before the compensatory drop commences (Fig. 2).

(3) If, instead of subjecting the fishes to KCN, while at these temperature extremes ( $10^{\circ}$  and  $30^{\circ}$ ), they were removed from these last and returned to an intermediate temperature before the test with cyanide, it was found that a complete reversal took place, the fishes of "warm" history now displaying a higher resistance than those of "cold" history, while the  $20^{\circ}$  fishes remained intermediate as previously. These reversed effects persisted clearly for 3 days after transfer to the intermediate temperature, less certainly after 5 and 10 days. (Table IV, Figs. 2, 3.) The re-reversal of the relations which was

TABLE III

Relation between duration of acclimatization in  $10^{\circ}$ , and resistance to KCN at the latter temperature. (Mean times in minutes).

Duration acclim.....	$\frac{1}{2}$ hr.-5 $\frac{1}{2}$ hrs.	1 day	3 days
Number of fishes.....	74	53	50
Length of resistance.....	$300.8 \pm 2.92$	$317.8 \pm 2.86$	$302.8 \pm 4.32$
Duration acclim.....	5 days	10-11 days	24-44 days
Number of fishes.....	10	26	33
Length of resistance.....	296.3	$289.1 \pm 3.81$	$285.5 \pm 5.58$

found at the end of 20 days (Fig. 2) may well be accidental, as are perhaps the exact relations of both of these curves to the "intermediate" ( $20^{\circ}$ ) line, which is based upon the mean figure for 100 fishes.

Certain relations of more or less certain significance are to be noted from inspection of Fig. 3. Thus, the curves for the "W" ( $30^{\circ}$ ) series, without exception, lie above those for the "C" ( $10^{\circ}$ ) series; the curves of each comparable pair (equal times in "I") diverge from one another, with increasing times of acclimatization; the terminals of the curves (with the exception of "I, 2 hrs." in the "W" series) are arranged in sequence, according to the length of subjection to "I" ( $20^{\circ}$ ), and are arranged in reverse order in the two series. The two "I, 2 hrs." curves, for example, are farthest apart, the "I, 10 days" ones closest together, etc. The various incongruities in the figure, as a whole, are doubtless due to the high individual variability shown by each set of fishes.



(4) It is probable that within broad limits no appreciable relation exists between size and susceptibility to KCN poisoning. In two experiments, 21 "large" and 17 "small" fishes were compared, the former averaging from 4 to 5 times as heavy as the latter. The mean times of succumbing were 101.0 and 103.4 minutes, respectively, there

TABLE IV

Persistence of effects of acclimatization to 10° ("C") and 30° ("W"), when followed by sojourns of varying length at intermediate temperature (20°) upon resistance to KCN at the latter temperature. Survival times in minutes. Values having high probability denoted by asterisks. For method of computing probabilities see Tippet, 1931, pp. 80-82.

Numbers in the two lots	Duration of acclimatization	Time in 20°	C	W	Difference (W-C)	Probability that difference is significant
29, 24	1 day	2 hrs.	89.7	104.4	14.7	*0.9999+
7, 10	1 "	1 day	90.7	107.2	16.5	*0.9959
10, 10	1 "	3 days	109.8	109.7	-0.1	0.5000
10, 10	1 "	10 "	100.5	101.2	0.7	0.5000
5, 5	3 days	2 hrs.	86.0	87.0	1.0	0.6500
5, 6	3 "	1 day	93.0	124.8	31.8	*0.9999+
15, 15	5 days	2 hrs.	90.5	115.5	25.0	*0.9999+
9, 10	5 "	1 day	90.8	121.3	30.5	*0.9999+
8, 10	5 "	3 days	101.4	116.0	14.6	*0.9985
10, 10	5 "	10 "	107.2	108.4	1.2	0.6300
11, 11	10-11 days	2 hrs.	83.8	107.0	23.2	*0.9999+
23, 18	33-40 d.	2 hrs.	84.2	123.2	39.0	*0.9999+
18, 20	36-51 "	1 day	92.0	129.0	37.0	*0.9999+
19, 20	36-56 "	3 days	98.5	127.0	28.5	*0.9999+
10, 10	57 days	5 "	101.7	110.9	9.2	*0.9969
18, 30	31-46 d.	10 "	111.9	116.7	4.8	0.8200
10, 10	46 days	20 "	110.7	103.6	-7.1	0.9245

being, in each of the experiments, an almost complete overlapping of the values.

#### BOILED SEA WATER

(1) When kept throughout the tests at the various experimental temperatures, the time relations of the deaths in boiled water were very similar to those in KCN though forming a less perfect logarithmic series (Table V, Fig. 1). The values for  $Q_{10}$  are here much less con-

stant, ranging from 2.3 to 3.0. These incongruities may be due to the considerably smaller number of individuals used.

(2) Death in deoxygenated sea water was more than 60 per cent slower, at the same temperatures, than in KCN solution. That this slower rate was not due to traces of oxygen left in our boiled water is known from the fact that considerable increases ( $\times 2$  or 3) in the small amount of oxygen present were not found to have any appreci-

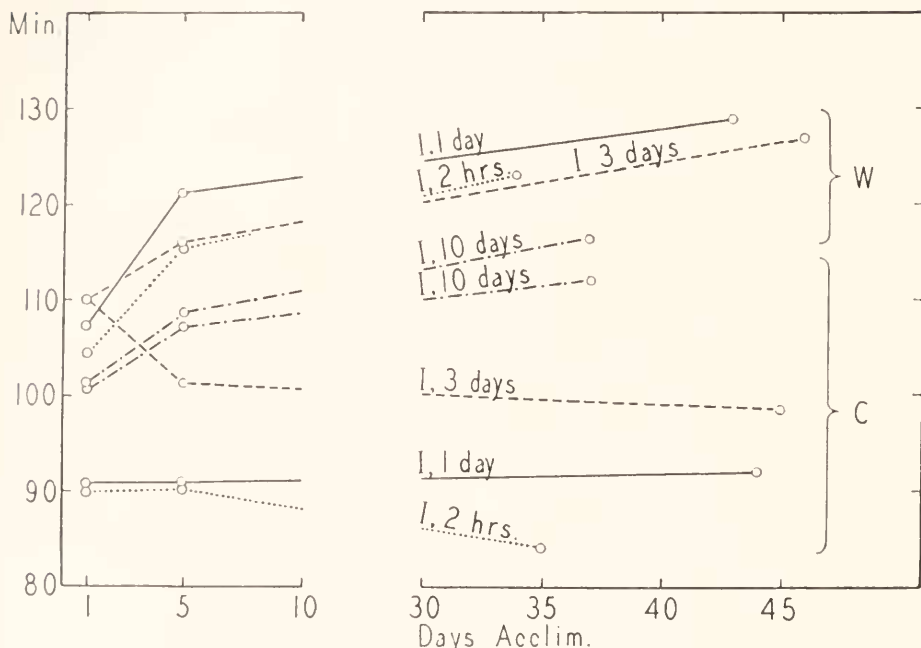


FIG. 3. Acclimatization for varying periods in "cold" ( $10^{\circ}$ ) and "warm" ( $30^{\circ}$ ), followed by sojourn for varying periods in "intermediate" temperature ( $20^{\circ}$ ), from which the fishes were originally taken. Abscissas = times of acclimatization in C and W (days). Ordinates = survival times in KCN solution (minutes). Separate curves are plotted for the various sets differing in their length of sojourn in I.

able effect. It may be due to the presence of a considerable reserve of oxygen in the tissues, which is rendered unavailable by the KCN. Or it may be that the latter has some toxic effect, independently of its effect upon oxygen utilization.

(3) Owing, in part at least, to the much smaller number of fishes used in the present series, the relations between duration of temperature conditioning and resistance to asphyxiation were not so well shown here as in the case of KCN. For those which had been acclimatized

for varying periods to 30°, a trend was nevertheless indicated, which agreed with that for KCN. This was not, however, true of the experiments at 10°.

(4) In two experiments in which "cold" lots were compared with "warm" or "control" (20°) lots, after transfer to the latter temperature, we had the same marked reversal as in the case of cyanide. Here, however, we had only 2 to 4 individuals of each sort.

(5) The size of the fishes, in experiments with boiled water, as contrasted with those in KCN or lethal heat, was found to have a marked influence upon the death-rate. Smaller (i.e. younger) fishes, as might have been expected (*for both KCN and boiled water*), were the first to succumb. Four experiments were performed, five fishes of

TABLE V

Mean times of survival in boiled sea water, at various temperatures, after 3 days acclimatization to these, and relative rates of metabolism on which "B.W." curve in Fig. 1 is based. For significance of figures for "relative metabolism" see Table I.

Number in each lot	Mean length (cm.)	Temperature of acclimatization (±)	Actual temperature in flasks during experiment	Times of survival		Relative Metabolism
				Mean	Range	
		°C.	°C.			
10	10.6	10	9.8	483.7	397-619	1.0
10	10.2	15	14.9	257.6	210-296	1.9
10	10.3	20	19.9	179.7	140-227	2.7
9	10.7	25	24.8	112.0	77-148	4.3
10	10.3	30	29.5	60.5	50- 77	8.0

each size being used in each of these. In two experiments, in which the mean weight of the large fishes was  $2\frac{1}{3}$  times that of the small ones, the average duration of life of the former was 15 per cent greater than that of the latter. In two other experiments, in which the large fishes averaged 4 times as heavy as the small ones, the difference was 41 per cent. The means for the 20 large and 20 small, comprised in the entire series were: 297.4 and 238.7 minutes respectively.<sup>6</sup> The rapid decline of respiratory metabolism with increasing size in fishes and other cold-blooded animals is now a familiar fact. (Keys, 1931, and Wells, 1935a, have presented data for fishes, based upon experiments conducted in this laboratory.)

<sup>6</sup> All four experiments yielded large mean differences, while in two of these there was no overlapping of values. But the four sets are not homogeneous enough in respect to temperature, etc. to justify the computation of probabilities.

## LETHAL HEAT AND COLD

(1) With fishes taken originally from a stock tank at 20°, differences in the length of conditioning to 30° led to marked differences in their subsequent resistance to lethal heat. Even a half hour's previous stay at 30° led to a considerable increase in resistance, and this increase continued, though at an ever-slowing rate, up to 10 (perhaps even 30) days, when this particular test was discontinued. After the first day, the rise of the curve was very gradual (Table VI, Figs. 4 and 5).

TABLE VI

Relations between length of acclimatization to 30° and resistance to lethal effects of heat, the resistance being indicated both by the time and the temperature at which the fishes succumbed. (Including only those experiments in which heat treatment commenced with 37.6±).

The probabilities relate to the differences between each temperature and the one in the preceding column. High probabilities indicated by asterisks.

Time of acclimatization	0	30 min.	1 hr.	2-2½ hrs.	4-5 hrs.	9 hrs.	15½ to 19½ hrs.
Number of fishes...	15	10	5	10	20	10	20
Mean time (minutes).....	4.9	10.6	21.5	24.5	30.1	34.4	40.9
Mean temp.....	37.73	38.03	38.36	38.48	38.73	38.89	39.06
Probabilities.....		*0.9999+	*0.9890	0.8740	*0.9999	*0.9940	*0.9999+

Time of acclimatization	1 day	3 days	10 days	30 days
Number of fishes.....	10	10	10	10
Mean time (minutes).....	41.5	53.6	61.0	62.2
Mean temp.....	39.11	39.32	39.62	39.68
Probabilities.....	0.9400	*0.9975	*0.9992	0.8300

Not included in the foregoing are three experiments in which the temperature of the "hot" tank at the outset was 38.75° (±), and in which the fishes under comparison (10 of each) had previously been conditioned to 30° for 3 and 39 days respectively. The mean lethal temperatures for the two lots were 39.50° and 40.11°, respectively, there being no overlapping of the figures for the contrasting lots.

(2) Return to a common temperature of 20° for considerable periods before testing with lethal heat diminished, but did not annul, the

effects of previous acclimatization. For example, a sojourn of 23 days at 20° did not suffice to eliminate the difference between lots which had been kept for 46 days at 10° and 30° respectively, while fishes kept for only a single day at these last temperatures retained a considerable part of the resulting difference after 10 days at 20°. On the other

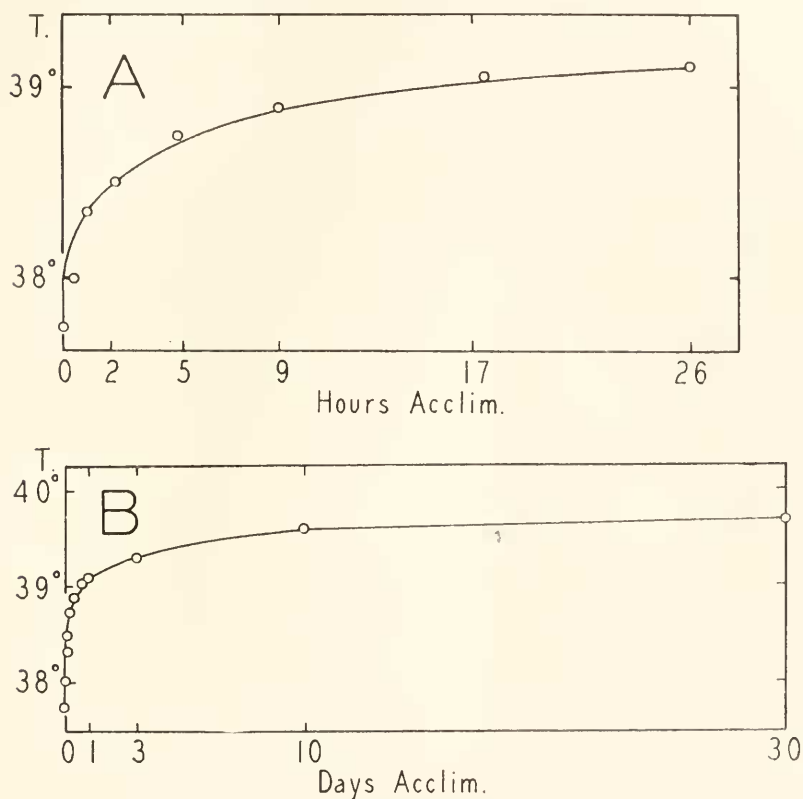


FIG. 4. Correlation between previous acclimatization in 30° tank and resistance to lethal effects of heat. Data for shorter periods only (up to one day) are plotted in A; those for both short and long periods are plotted, on different scale, in B. Abscissas = times of acclimatization in hours (or days); ordinates = mean temperatures which proved lethal to the various lots.

hand, the effects of 3 days conditioning at these extreme temperatures had nearly or quite disappeared after 30 days at the intermediate one. This last is not in agreement with certain results reported for *Fundulus* by Loeb and Wasteneys (1912), who state: "The immunity against a temperature of 35° acquired by keeping the fish for two days at 27° is not lost or weakened if the fish are afterwards kept as long as thirty-

three days at a temperature of from  $10^{\circ}$  to  $14^{\circ}$ ." It may well be that *Gillichthys* differs from *Fundulus* in this respect. These authors likewise state: "The immunity against a temperature of  $35^{\circ}$  C. is also main-

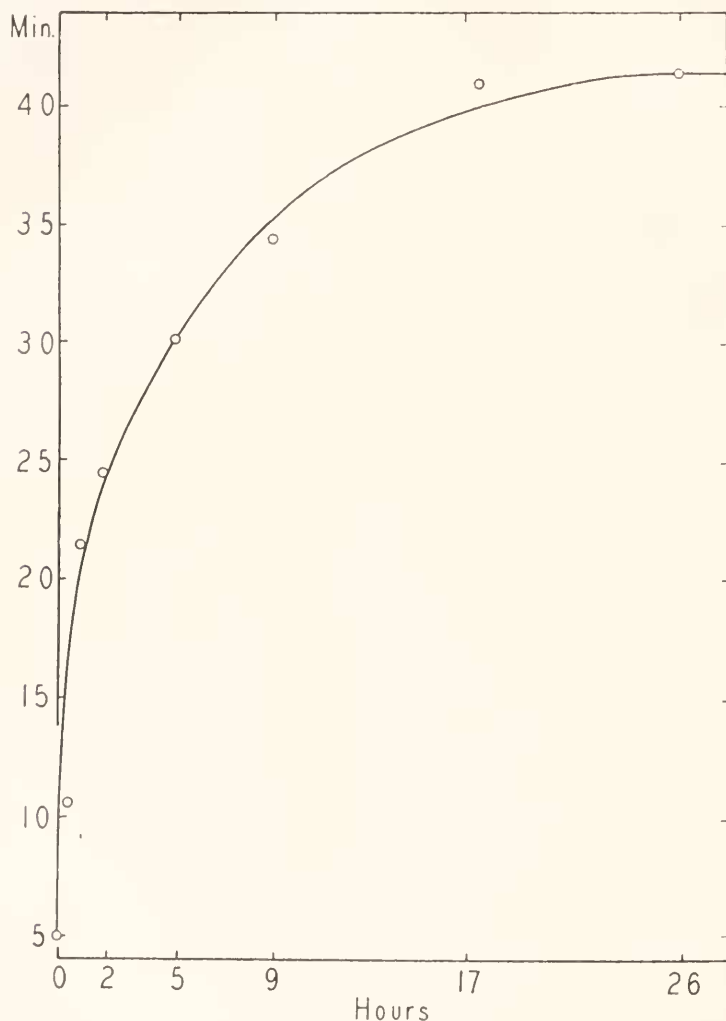


FIG. 5. This is the counterpart of Fig. 4A, the present values representing the mean times in which death occurred, instead of the temperatures.

tained if the fish are kept after the two days' exposure to  $27^{\circ}$  for two weeks at a temperature of  $0.4^{\circ}$  C." We have not employed such a low temperature in this connection.



Table VII<sup>7</sup> comprises the chief results of experiments of this class, while Figs. 6 and 7 depict, for one series of tests, the relations between the periods of conditioning in "cold" and "warm" water (10° and 30°) and the subsequent sojourn at the "intermediate" temperature (20°). The divergence of the two sets of curves in Fig. 6, and their

TABLE VII

Persistence of effects of acclimatization to 10° ("C") and 30° ("W"), upon heat resistance, when followed by sojourns of varying length at intermediate temperature (20°).

The probabilities relate to differences between lethal temperatures for C and W lots. High probabilities indicated by asterisk.

Number of fishes in each lot	Period of acclim. (10° and 30°)	Subsequent stay at 20°	Temp. of hot tank at start	Period of resistance		Lethal Temp.		Probability
				C	W	C	W	
10 (9 in W)	6 hrs.	2(+) hrs.	36.0°	23.0	55.8	37.38°	38.56°	*0.9620
10	1 day	2½ hrs.	36.0°	15.1	67.1	36.96°	39.09°	*0.9999+
10	" "	1 day	36.0°	28.7	61.3	37.50°	39.11°	*0.9999+
10	" "	3 days	36.0°	26.7	53.3	37.58°	38.68°	*0.9999+
10	" "	10 "	36.0°	22.2	36.3	37.31°	38.02°	*0.9997
15	3 days	30 days	36.0°	18.8	20.4	36.99°	37.07°	0.6900
10	5 days	2 hrs.	37.0°	5.0	58.3	37.30°	39.27°	*0.9999+
10	" "	1 day	36.0°	29.0	70.1	39.64°	39.53°	*0.9999+
10	" "	3 days	36.0°	13.5	64.7	36.80°	39.11°	*0.9999+
10 (9 in W)	" "	10 "	36.0°	18.9	47.4	37.25°	38.66°	*0.9999+
10	34 days	2(+) hrs.	37.0°	2.5	70.1	37.20°	39.89°	*0.9999+
10	37 "	1 day	36.0°	5.7	80.9	36.39°	40.09°	*0.9999+
10	" "	3 days	36.0°	7.4	76.3	36.54°	39.85°	*0.9999+
10	32 "	10 "	36.0°	12.7	53.7	36.82°	38.90°	*0.9999+
10	46 days	23 days	36.0°	14.1	37.6	36.87°	38.23°	*0.9999+
23‡	" "	47 "	36.0°	24.7	27.9	37.36°	37.55°	0.8850

‡ In this experiment, 10 "controls" which had been kept continuously at 20° gave mean values: time, 18.3 minutes; temperature, 37.07°.

convergence in Fig. 7 represent significant facts, as do the relative positions of all of the single curves in Fig. 7 and some of those in Fig. 6.

(3) Long-time (38-day) conditioning of fishes to a low temperature (10°) made them more resistant to extreme cold (1.0° —) than

<sup>7</sup> Corroboration of some of these results was obtained from a number of experiments which could not conveniently be included in the table.

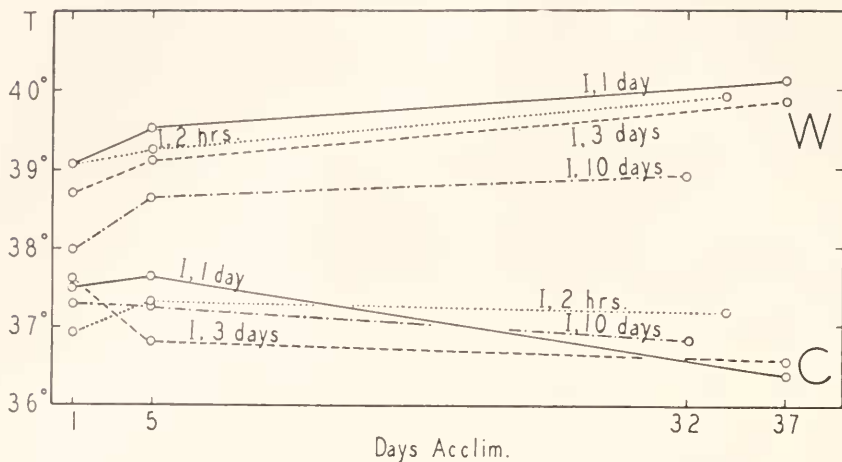


FIG. 6. Same legend as for Fig. 3, except that the present figure relates to heat resistance, the ordinates representing temperatures which proved lethal to the various lots of fishes.

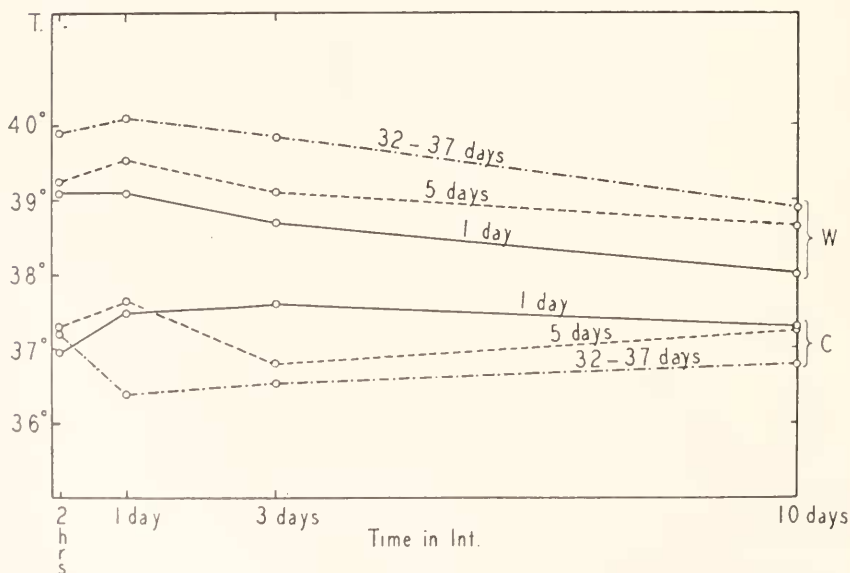


FIG. 7. Based upon the same experiments as Fig. 6, but with different treatment of the data. In the present case, the subsequent sojourn in "intermediate" ( $20^{\circ}$ ) is plotted on the abscissas axis, while separate curves are drawn for the various periods of previous conditioning to "cold" and "warm."

ones which were transferred to 10° only 3 days previously. Our evidence here is reasonably conclusive, though not lending itself readily to quantitative treatment (see pp. 407-8).

(4) Fishes kept alternately at 10°, 20° and 30° for 7 or 8 com-

TABLE VIII

Effects upon resistance to heat of previous subjection to "cold" (10°), "intermediate" (20°) and "warm" (30°), in alternation.

Temp. of hot tank at start	Rate of rise	Number of fishes	Temperature history	Period of resistance (minutes)	Lethal temperature
37.6°-37.7°	0.1 in 13 min.	8	5 cycles: W(24 hrs.), I(12 hrs.), C(24 hrs.), I(12 hrs.), etc.	32.7	
		8	5 cycles: C(24 hrs.), I(12 hrs.), W(24 hrs.), I(12 hrs.), etc.	46.0	
		8	Control: I (continuously)	4.5	
37.0°-37.1°	0.1° in 2.8 min.	10	8 cycles: W(12 hrs.), I (12hrs. ), C(12 hrs.), I(12 hrs.), etc.*	29.5	38.20°
		10	7 cycles: W(12 hrs.), I(12 hrs.), C(24 hrs.), I(12 hrs.), etc.†	42.5	38.47°
		20	Control: I (continuously)	6.0	37.30°
			Probability of difference between first and second temperature low (0.8100); between first and third very high (0.9999+)		
36.0-36.1	0.1° in 2.1 min.	10	9 cycles: W(12 hrs.), I(12 hrs.), C(24 hrs.), I(12 hrs.), etc.†	66.7	39.25°
		11	Control: I (continuously)	15.1	36.91°
			Probability of difference very high (0.9999+)		
37.6	0.1 in 2.0 min.	15	W (6 days, continuously)	42.8	39.80°
		15	W and I (12-hr. periods alternately for 12 days)	43.0	39.79°
		15	W and I (2-day periods alternately for 12 days)	43.0	39.79°

\* Except that last C and W were each 24 hours.

† Thus C time = 2 × W time.

plete cycles were more resistant to heat than ones which had been kept continuously at 20°. This was decisively true, even in some experiments in which the "cold" periods were twice as long as the "warm" ones (Table VIII).<sup>8</sup> On the other hand, fishes which had been sub-

<sup>8</sup> A somewhat similar experiment was performed by Loeb and Wasteneys (1912), with results similar to ours.

jected to these alternations of temperature were *less* resistant to extreme cold ( $0^{\circ}$ — $1^{\circ}$ ) than ones kept continuously at  $20^{\circ}$ . This statement is based upon four experiments, involving 28 "alternation" fishes and 19 controls. While the results cannot well be treated quantitatively, for reasons already stated, this general statement admits of little doubt.

(5) When the water temperature was raised gradually, throughout a period of several hours, the tolerance was considerably increased, in comparison with experiments in which the fishes were subjected abruptly to these higher temperatures. Thus when 15 fishes were placed in a tank at  $32.2^{\circ}$  and the water gradually warmed, the first death occurred when the temperature reached  $37.6^{\circ}$ . This was  $5\frac{1}{2}$  hours after the commencement of the experiment, and  $1\frac{1}{4}$  hours after the temperature had reached  $37.2^{\circ}$ . On the other hand, when 19 fishes of the same original stock were placed abruptly in water at stationary temperatures of  $36.9^{\circ}$  to  $37.2^{\circ}$ , they all died within a maximum of 17 minutes, the mean time being 10 minutes.

(6) In four experiments, comparisons were made of the resistance to heat of large and small individuals (totalling 21 of each), having identical temperature history. The former averaged about four times as large, by weight, as the latter. The mean temperatures at which they succumbed averaged: large,  $37.79^{\circ}$ ; small,  $37.48^{\circ}$ . Comparison of individual figures makes it highly improbable that any relation exists between size and resistance to heat, or at least any which could be revealed without the use of much greater numbers of fishes.<sup>9</sup> This is in marked contrast to the consistent differences which were generally to be observed in comparing fishes of different temperature history.

(7) In a single experiment, the question was tested whether resistance to heat was affected by the salt concentration of the water. Unlike Loeb and Wasteneys (1912), we used for this test a fresh water species (*Gambusia affinis*). This fish can be acclimatized to 75 per cent sea water (probably to full strength) if the change is made in several steps. In our single experiment, two lots, of 17 and 16 fishes respectively, which had been kept in fresh water and 75 per cent sea water (the latter in this medium for 5 days), were subjected to lethal heat. The mean temperature of succumbing of the fresh water individuals was  $37.46$ , that of the salt water ones  $38.53$ . If one individual were removed from each series, there would be no overlapping between the two. Thus heat was more rapidly fatal to this fresh water species

<sup>9</sup> Bělehrádek (1935) cites contradictory figures as regards the relation of age to heat-resistance in fishes. M. M. Wells (1914), on the other hand, on the basis of experiments upon several species of fresh-water fishes, concludes: "Large fish of a given species are more resistant to high temperatures than small fish of the same species." Wells used *Ameiurus melas* and several species of *Notropis*.

*in fresh water*, than in the unfamiliar medium to which it had been rather rapidly acclimatized.

#### URETHANE

(1) In comparing the times required for anesthetizing at different temperatures, no consistent results were obtained. The earlier inference (Sumner and Wells, 1935) that the effects of urethane were directly related to the rate of respiratory metabolism does not appear to be well founded.

(2) Nevertheless, when acclimatization at 10° and 30° was followed by sojourn at a common temperature (20°), tests with urethane, made at the latter temperature, gave consistent results similar to those reported by Sumner and Wells, and agreeing with those reported in the present paper for KCN and boiled water. Fishes of previous "warm" history were much more resistant than ones of previous "cold" history, and these differences tended to be great in proportion to the duration of the previous acclimatization.

(3) When comparison was made of fishes (10 each) which had been kept at 30° for 5 hrs., 1 day, 3 days and 34–35 days, a consistent increase in resistance was manifested, the last set requiring nearly three times as long for anesthetization as the first.

(4) No consistent results were obtained when similar tests were made at 10°.

This last fact is due, in part, to the difficulty in finding a satisfactory "end-point" for urethane anesthesia at low temperatures. For this and other reasons, we abandoned the plan to make more extensive tests with this drug. While the results stated in paragraphs 2 and 3 of this section appear to represent actual physiological differences, we are not disposed to attempt any interpretation of these at present.

#### RESPIRATORY MOVEMENTS

(1) The frequency of respiratory movements, based upon 45 "cold" (10°) fishes, 78 "intermediate"<sup>10</sup> (20°) ones, and 44 "warm" ones, was  $26.4 \pm 0.61$ ,  $73.7 \pm 1.15$ , and  $74.8 \pm 1.95$ , respectively, per minute. The figures upon which these averages are based are restricted to experiments in which fishes at all three temperatures were available for simultaneous comparison. It is unlikely that the "warm" and "intermediate" fishes differed significantly (Table IX).

<sup>10</sup> This number included many of the "warm" and "cold" fishes, which were tested at 20°, before subjection to the other temperatures.

(2) Transfer from 20° to 10° resulted in a mean fall in respiratory rate of  $39.7 \pm 2.02$  per minute (mean of 39 fishes). After return to 20°, this was followed, in the 37 fishes thus tested,<sup>11</sup> by a mean rise of  $32.5 \pm 1.34$  per minute.

(3) Transfer from 20° to 30° resulted, quite unexpectedly, in no consistent rise in respiratory rate. In seven experiments (3 fishes each), in which counts were made of the breathing of fishes that had been transferred to the 30° tank from 2 hours to 4 days previously,

TABLE IX

Mean rates of respiratory movements at 10° ("C"), 20° ("I") and 30° ("W")

Duration acclim. in C and W	Temp. history	Number of fishes	Mean length (cm.)	Resp. per minute
3-4 hrs.	C	6	12.7	36.0
	I	18	12.7	73.5
	W	6	12.8	86.7
18-24 hrs.	C	12	13.1	27.5
	I	30	13.0	73.2
	W	12	13.3	70.8
2 days	C	9	13.1	26.1
	I	24	13.0	72.6
	W	8	12.7	74.8
10-11 days	C	12	13.2	22.4
	I	12	13.0	76.6
	W	12	13.2	81.2
33-37 days	C	12	13.7	29.4
	I	12	14.3	74.4
	W	12	13.9	72.5
Means (omitting 3-4 hours)	C	45	13.29	$26.4 \pm 0.61$
	I	78	13.20	$73.7 \pm 1.15$
	W	44	13.33	$74.8 \pm 1.95$

the mean rise in respiratory rate was a nominal one (1 per minute), there being actually more negative cases than positive ones. On the other hand, there was a large and consistent *fall* when 30° fishes were returned to 20°, the average difference for 28 fishes being  $33.3 \pm 2.50$  per minute. These two sets of facts are difficult to reconcile with one another.

(4) When fishes of "warm" and "cold" history (28 of each)

<sup>11</sup> Mostly different individuals from those covered by the statement immediately preceding.



were compared 15 minutes to 2 hours after transfer to 20°, the latter gave a distinctly higher rate, this difference averaging  $15.9 \pm 2.73$  per minute. Here we had the same reversal of relation as was found with KCN and boiled water.

(5) The rate of respiratory movements was dependent to some extent on size. In each of 4 experiments, 5 "small" and 5 "large" fishes were used, the temperatures being 15.1° to 15.5°. The 20 small ones, having a mean weight of about 34 grams, gave a mean rate of 49.1 respirations per minute, as compared with 26.9, for the 20 large fishes, which averaged about 73 grams. In these four experiments, only a single case of overlapping occurred between the two contrasted lots.

### DISCUSSION

From the various quantitative relations revealed in our tables and curves, it is conceivable that definite conclusions might be drawn regarding the nature of the various chemical reactions concerned. The present writers do not feel disposed, or indeed qualified, to undertake any such general interpretations of our results. It may, however, be instructive to point out analogies between certain features of these results and some other biological phenomena.

The situation depicted in Fig. 2 seems to find an instructive counterpart in the field of sensory physiology. The intensity of a sensation rises rather abruptly to a maximum, following a stimulus, and then falls again, even while this stimulus is continued. The crossing of the two curves in our figure, following the return of the two sets of fishes to a common, intermediate temperature, recalls an old and familiar experiment in sense physiology. We refer to the one in which the subject's two hands are dipped into hot and cold water, respectively, and then, after a few minutes, they are removed and dipped simultaneously into water of medium temperature. One experiences a reversal of his temperature perceptions quite parallel to the reversal in metabolic rate which is manifested by the fishes after a similar transfer. Such an analogy is not, of course, to be offered as a substitute for an explanation in physico-chemical terms. But in the absence of a satisfactory explanation of the latter type, it may have suggestive value. Indeed, it seems likely that this analogy is based upon fundamental similarities in the irritability of quite different sorts of living matter.

The relations shown in Fig. 1 are representative of a wide range of phenomena which have to do with the effects of temperature upon the rates of vital processes. (Cf. Krogh, 1916; Bělehrádek, 1935.) The vast and well-tilled field of "temperature coefficients" (cf. Běleh-

rádek, 1935, Heilbrunn, 1937) is one which we have no intention to discuss.

Our curves (Figs. 4 and 5), are quite definite in their trend, though we doubt whether they furnish anything very distinctive by which the underlying chemical or physical processes could be identified. Perhaps others might find this possible. It hardly seems likely, however, that such a rapid acclimatization to heat could result from the production of fats having successively higher melting-point (Heilbrunn, 1937; Bělehrádek, 1935), though we should not be warranted in denying this possibility. Biochemical processes in the field of sense physiology are known which are even more rapid than this, e.g. light- and dark-adaptation in the human eye (Hecht, 1920, Tansley, 1931). The striking similarity between our curves (Figs. 4 and 5) and some of those offered by the latter writer perhaps deserves mention. Tansley calls attention to the close parallel between the rate of a sensory change, determined subjectively, and a measured biochemical change (restoration of visual purple), which is presumed to be intimately associated with this. Some future physiologist may be able to prove that heat-acclimatization rests upon an equally simple chemical transformation.

Regarding the much-discussed question of the cause of death at (moderately) high temperatures, we can make no contribution of a positive nature. We can, however, offer reasons for doubting the adequacy of one of the agencies which have been proposed, namely a fatal rise in the rate of respiratory metabolism. We have already called attention to the difference between the effect of temperature conditioning upon resistance to heat and its effect upon resistance to asphyxiation by boiled water or by cyanide.

The former effect was of far greater magnitude than the latter. Resistance to heat, as measured by survival time, increased eight-fold in the course of one day, and continued to increase quite appreciably for some days further. Resistance to KCN, measured in the same way, increased about 50 per cent in the first day, and any further increase was only detectable statistically. Increase of resistance to asphyxiation in boiled water was even less obvious than in the case of KCN, though this last fact may have been due to the smaller numbers of fishes which we employed.

There were equally striking differences in the *persistence* of the effects of heat-conditioning upon resistance to heat and to asphyxiation. The effect upon heat resistance was very much more lasting, after withdrawal of the original temperature conditions, than was the effect upon resistance to KCN. (Boiled water was not tested in this connection.)

Still another difference of interest, already mentioned, concerned the relation of size to susceptibility to these lethal agents. As regards heat resistance, no difference was found, within rather broad limits. In boiled water, on the other hand, the smaller fishes rather uniformly died earlier, as might have been expected. Quite unexpectedly, however, no such difference was found in experiments with KCN. For this we can offer no present explanation.

It would appear, therefore, that there are at least two distinct processes involved in acclimatization to a higher temperature: (1) a regulative decline in the initially much increased respiratory metabolism; and (2) another change of an unknown nature (either physical or chemical), by which the threshold of susceptibility to destructive temperature is raised.

#### SUMMARY

Fishes (*Gillichthys mirabilis*) having different temperature histories were subjected to KCN, to boiled sea water, and to temperatures high or low enough to be lethal. Urethane, an anesthetic, was used in a limited number of experiments. The time of death (or of anesthetization) was recorded for each of the fishes (nearly 3,000). Counts of respiration were made in a considerable number of cases.

Death in both KCN and in boiled water was speedy in proportion to the temperature, the reciprocals of the times of death forming an approximate logarithmic series when plotted against temperature. The temperatures used were 10°, 15°, 20°, 25° and 30°.

In the case of KCN, a low correlation was found to exist between the duration of previous acclimatization to high or low temperatures and the degree of resistance to KCN at those temperatures. The abrupt decrease of resistance (increase in metabolic rate), resulting from transfer to a higher temperature, was followed by a slight increase of resistance which continued for several days. On the other hand, the abrupt increase of resistance (decrease in metabolic rate), resulting from transfer to a lower temperature, was followed by a slight decrease which continued for several days.

In boiled water, this compensatory trend was only shown after transfer to higher temperatures.

When fishes which had been acclimatized to high (30°) and low (10°) temperatures were tested in KCN, boiled water or urethane at 20°, there was a consistent reversal in the resistance of the respective lots, the 30° fishes now being much more resistant than the 10° ones. These relations continued for several days after transfer to the intermediate temperature.

Death in de-oxygenated sea water was more than 60 per cent slower, at the same temperatures, than in KCN solution.

Acclimatization at 30°, even for brief periods, resulted in a marked resistance to the lethal effects of heat. This result was already conspicuous, after a half hour at 30°, and increased, at a diminishing rate up to 10 days or more.

Return to a common temperature of fishes having "cold" and "warm" histories diminished, but did not annul, the effects of previous acclimatization upon heat resistance. Pronounced differences were noted in one experiment, after 23 days at the intermediate temperature. Interesting relations were pointed out between the period of conditioning and the persistence of the effects.

Acclimatization at 10° increased resistance to lethal cold (1.0°—).

Fishes kept for alternating periods at 10°, 20°, and 30° (even when the 10° periods were twice as long as the 30° ones) were more resistant to lethal heat than fishes kept continuously at 20°. Such fishes were, however, less resistant to lethal cold than the controls.

The rate of the visible respiratory movements was much greater at 20° and 30° than at 10°. There was no certain difference, however, between the rates at the first two temperatures.

Transfer of fishes of "cold" and "warm" history to an intermediate temperature resulted in the same reversal in relative respiratory rates as was found in the case of resistance to KCN, etc.

The size of the fish appears to have little or no influence upon its resistance to lethal heat or to cyanide poisoning. On the other hand, it was found that smaller fishes had a more rapid respiratory rhythm, and succumbed more rapidly in boiled water than larger ones.

Acclimatization to higher or lower temperatures brings about two classes of effects which seem to be largely distinct from one another. One of these concerns increased resistance to the harmful effects of heat and cold, when these are used as lethal agents. The second of these concerns changes in respiratory metabolism, and therefore in resistance to oxygen lack, including KCN poisoning. Reasons are given for believing that the former effect is not dependent upon the latter.

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